membranes (BLMs), which are freely suspended over a micro machined aperture in an aqueous solution. This new way of membrane structure analysis allows investigating bio molecular and organic sub-stances in aqueous environments by parallel and divergent beam propagation imaging, using partially coherent multi-keV x-ray radiation. The width of the thinning film is significantly smaller than the detector pixel size, but can be resolved from quantitative analysis of the intensity fringes in the Fresnel diffraction regime down to its native thickness of about 5nm. We have put forward a simplified but extendable model, which enables the theoretical description of image formation and charac-terization of membrane thickness and its decrease during the thinning process from a bulk to a bimolecular film. The structural changes can be obtained from both the loss of contrast and the asymmetry of the detected Fresnel fringes. On the basis of the recent experiments, future investigations will be performed to study the inte-ractions of membranes, as they are for example known from synaptic fusion, with high spatial resolution.

1836-Pos Board B746
Characterization of Lipid Asymmetry in Plasma Membrane-Derived Vesicles
Lirong Chen, Kalina Hristova.
Plasma membrane-derived vesicles are a good model system for studies of membrane protein interactions in mammalian membranes. In this project, our goal is to characterize the lipid asymmetry in the vesicles over time. The phospholipid distribution in the plasma membrane is asymmetric. Phosphatidylserine (PS) is predominantly found in the inner leaflets of the intact plasma membrane. Exposure of PS on the outer leaflet of the plasma membrane can be detected by annexin V, which preferentially binds to negatively charged phospholipids like PS. An assay is developed to study the lipid asymmetry in plasma membrane-derived vesicles using fluorescently labeled annexin V. Cells are vesiculated and FITC labeled annexin V is added to vesicles at different time points, and imaged using a confocal microscopy. A matlab program is developed to quantitatively analyze the intensity of FITC on the vesicles. As a control, the vesicles are scrambled completely by subjecting to freeze-thaw cycles and imaged. The results suggest that lipid asymmetry is lost during the vesiculation process.

1837-Pos Board B747
Electron Cryo-Tomography of Cilia-Associated Structures of Rod Photoreceptors
Jared C. Gilliam, Juan T. Chang, Wah Chiu, Theodore G. Wensel.
Vertebrate rod photoreceptors sense light with modified primary cilia known as rod outer segments. The regions of the cell surrounding the junction between the inner and outer segments, known as the connecting cilium or transition zone, are the sites of highly active biosynthetic activity, directional trafficking, and sorting of protein and membranes. Defects in the cellular machinery associated with these processes give rise to numerous blinding diseases, including the retinal ciliopathies, but their structures and compositions are poorly understood. The transition zone and adjacent structures are ideal subjects for electron cryo-tomography because the entire connecting cilium is thin enough for imaging over a wide range of tilt angles, and because this region contains many structures with contiguous surfaces and functional units that can be identified, including disks, endoplasmic reticulum, mitochondria, vesicle and plasma membranes, microtubule bundles of the axoneme and basal bodies, ribosomes, and the ciliary rootlet. We have generated multiple 3D reconstructions from tomograms of this region of mouse rods, and quantified the recurring features of these structures. The results reveal for the first time in quantitative detail the three-dimensional structure of cilia-associated machines in a mammalian neuron, without distortions caused by fixatives, stains and sectioning. One surprising feature observed is a lack of continuity between basal disks and the surrounding, non-invaginated plasma membrane.

1838-Pos Board B748
A study of Functional ion Transport Using Tethered Membranes
Bruce A. Cornell, Andrew Battle, Louise Brown, Sonia Carnie, Sophia C. Goodchild, Hedayetul Islam, Donald Martin, Boris Martinac, Russell Richards, Stella Valenzuela.
Tethered membranes (t-BLMs) are lipid bilayers that are chemically attached to a solid surface. In the present studies the surface is gold on polycarbonate and the attachment chemistry, sulphur to gold. t-BLMs are more stable making possible longer and more complex experiments of model and biomembranes. t-BLMs have been used here to study the ion conduction properties of both native and mutant forms of four ion channels families including: the Na^+/H^+ co-transport protein; the Voltage Dependent Anion Channel transporter (VDAC); the Chloride Intracellular Channel Family (CLIC); the Mechano-Sensitive Channels (MscL) and (MscS). Of the four channel types CLIC spontaneously inserts into the t-BLM, the MscL and MscS were transferred via a detergent rinse, and the VDAC and co-transporter families were transferred from proteoliposomes. A novel mixed tethered/mobile t-BLM system was used to overcome previous limitations impeding the insertion of ion channels into tethered membranes and overcoming the poor electrical seals which previously prevented the observation of the ion conductance of the inserted ion channels. The successful approach employs a mix of tethered and mobile lipid species. Tether densities in the range 10%-40% were used, permitting the incorporation of intrinsic membrane proteins with molecular weights in the range 15-50kDa into the untethered free lipid patches. The results of these studies will be compared with results for the same proteins studied using classical patch clamp techniques.

1839-Pos Board B749
Giant Protein Vesicles for Studying Membrane Proteins
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Giant vesicles offer a great potential as a native like cell-mimetic environment for protein reconstitutions and subsequent protein characterization. However, protein reconstitution into giant vesicles is not straightforward and the assembly procedures of giant vesicles generally preclude protein reconstitution. This has so far limited the use of these vesicles as a general biomimetic platform for studying membrane proteins and in novel membrane protein-based biotechnological applications.
In this study we present a method for formation of giant protein vesicles (GPV). The method supports formation of GPV in physiological ionic strength buffers such as phosphate buffered saline, and does not require specialized equipment, specialized lipids and peptides or a dehydration/rehydration step. Moreover, the amount of reconstituted protein in the GPV may be controlled by the lipid-to-protein ratio of the large vesicles. We characterized GPV formation using the spinach aquaporin SoPIP2;1 and *E. coli* aquaporin AqpZ as model proteins of α-helical polytopic membrane proteins. Functional protein reconstitution into GPV is demonstrated with the light-driven proton pump Bacteriorhodopsin.
We suggest that the described method may constitute a general and versatile method for the formation of GPV. This may open up for the possibilities of a more general use of membrane proteins in biotechnological applications including membrane protein biosensors, drug discovery, bioreactors, nano-machines and novel separation technologies.

1840-Pos Board B750
Flexible Surface Model Explains Time-Resolved Uv-Visible Studies of Rhodopsin Activation in a Membrane Lipid Environment
James W. Lewis, Istvan Szundi, David S. Kliger, **Michael F. Brown**.
Rhodopsin is the quintessential GPCR–the UV/Visible absorbance of its bound chromophore provides detailed time-resolved information about the GPCR activation steps [1]. At least four species equilibrate on the millisecond time scale after rhodopsin photoexcitation in a membrane environment [2]. The first millisecond-time scale equilibrium is between the protonated Schiff base (PSB) species Meta I_{480} and the deprotonated SB species Meta IIa and is pH independent. Subsequent equilibria involve spectrally silent transitions of Meta IIa to produce Meta IIb and protonation of Glu134 of the E(R)Y sequence in Meta IIb to give Meta $IIbH^+$ where pK_a describes the acid-base equilibrium. We used time-resolved absorbance measurements on the microsecond-to-hundred millisecond time scale to study effects of the membrane lipid environment on the first equilibrium constant, K_1, and on the pK_a of the final equilibrium. Reconstituted membranes of rhodopsin with POPC, DOPC, or DOPC/DOPE mixtures were studied at 30°C. Results were analyzed by singular value decomposition and globally fit to a sum of exponential terms. We discovered a striking increase in K_1 due to either PE head groups or increased acyl chain unsaturation; by contrast pK_a changed little. According to the flexible surface model (FSM), there is competition between the curvature elastic energy and the hydrophobic mismatch at the proteolipid boundary that explains the above influences of lipid-protein interactions [3]. The fact that both the lipid acyl chains and polar head groups affect the Meta I-Meta II transition of rhodopsin is a striking illustration of how protein energetics in membranes are governed by chemically nonspecific properties of the lipid bilayer. [1] J. Epps *et al.* (2006) *Photochem. Photobiol.***82**, 1436-1441. [2] E. Zaitseva *et al.* (2010) *JACS***132**, 4815-4821. [3] A.V. Botelho *et al.* (2006) *BJ***91**, 4464-4477.